

***Bordetella* species**

Includes *B. pertussis* (whooping cough)
(615) 262-6362

Introduction

Specimens for isolation of *Bordetella pertussis* in suspected cases of whooping cough are accepted from public and private health care providers. Only **symptomatic** contacts of diagnosed cases of pertussis will be accepted for examination, since a carrier state in asymptomatic contacts has not been demonstrated as an important source of transmission. Reference cultures are accepted for confirmation of *Bordetella pertussis*, *B. parapertussis*, and *B. bronchiseptica*. *B. pertussis* and *B. parapertussis* are identified by culture. *B. bronchiseptica* is identified by culture techniques. *B. pertussis* is also identified by Polymerase Chain Reaction (PCR).

Specimen Collection for Testing for Whooping Cough

Collect nasopharyngeal swabs as soon as possible after the onset of symptoms and before antibiotic treatment is started. There is a greater likelihood of positive cultures in the first two weeks of symptomatic infection than during the later weeks of illness.

A kit containing the materials and instructions necessary for collecting a nasopharyngeal specimen is available from the Tennessee Department of Health (TDH) Laboratory. Do not order more kits than are needed. They contain culture medium with a short shelf life.

The kit contains:

- Instructions for collection of specimens
- 2 Dacron nasopharyngeal swabs (one for PCR, one for culture)
- 2 tubes of Regan-Lowe transport medium
- 1 Form PH-1573 & 1 Mailing label

Collection Procedure

1. To obtain the specimens, immobilize the patient's head and pass the swab through the nostril completely to the back of the **nasopharynx**, and leave it in place for at least 5 seconds before withdrawing. If entry cannot be gained through either nostril, enter through the mouth; however, the **throat area provides an inferior method for culture**.
2. **Obtain 2 swabs.**

Specimen Processing (Refer to Figure II - 1, Page II - 21, FLOW CHART FOR THE COLLECTION OF BORDETELLA PERTUSSIS SPECIMEN.)

After collecting the samples:

1. Place one swab into a labeled Regan-Lowe transport medium for PCR. Replace the cap with the swab still in the tube. Keep cold at 4°C.
2. After collecting the second specimen, immediately place the swab in the second Regan-Lowe transport. Replace the cap with the swab still in the tube. Keep cold at 4°C.
3. Label the Regan-Lowe transport medium tubes with the patient's name.
4. Complete the Miscellaneous form PH-1573. No culture confirmations or PCR will be performed without a completed form accompanying each specimen. Return completed form PH-1573 to the mailing container

Bordetella (Continued)

Keep the Regan-Lowe transport medium, containing the swab, at 4°C while held at the clinic and during transport.

If specimens cannot be sent or transported to laboratory immediately, **refrigerate at 4°C**. Do not hold longer than 24 hours; specimens must reach the laboratory as soon as possible after collection.

Specimen Preparation - Reference Cultures

Subculture isolated organisms for identification or confirmation to appropriate media slants, and incubate until growth is apparent. If tubes are not available, cut out a chunk of the plating media with good growth and place it in a sterile tube.

Do not mail reference cultures on plates. Plates are acceptable only if a courier delivers them directly to the laboratory.

Specimen Identification

1. Complete all the provider and patient information areas on the Miscellaneous Exam Form PH-1573. Submit the following information: nature of symptoms, date of onset of symptoms, immunization history, and contact with other cases of whooping cough, any antibiotic therapy before specimen collection, and other pertinent information. Include biochemical information with reference cultures.
2. Using indelible ink, label each specimen (transport medium) with the date of collection and the patient's first and last name. Attach the control number on the tear strip to the transport medium specimen, and secure it with transparent tape. Unlabeled specimens, or specimens where the patient identifier on the specimen does not exactly match the identifier on the form, **will not be tested**.

Shipment of Specimens

1. Wrap the transport medium in absorbent material. Pack the specimens in the pertussis mailing container provided by the TDH Laboratory. Packing and shipping specimens to the state public health laboratory requires personnel trained in current regulations. Follow the shipping guidelines of your current carrier or method of shipment.
2. Affix the mailing label (PH-0838), return address, and infectious substance (etiologic agent) or clinical (diagnostic) specimen label to the outer mailing container.
3. Ship the specimens to the Tennessee Department of Health Laboratory in Nashville.
4. Use first-class postage on US mail.
5. Contact the Aerobic Bacteriology Section if an outbreak is suspected and specimens will be submitted.

Bordetella (Continued)

Reporting Procedures and Interpretation of Results

Bordetella pertussis - Results are reported when the culture results are complete within 7 to 10 working days after the specimen is received. Positive culture results for *B. pertussis* are also reported by telephone to the Tennessee Department of Health Immunization Program.

Cultures are reported
No <i>Bordetella pertussis</i> isolated.
Positive for <i>Bordetella pertussis</i> .

Culture examinations may fail to detect *B. pertussis*. As the disease process may continue for weeks or months after viable organisms no longer remain in the nasopharynx, a negative culture report does not rule out infection, especially if the specimens were collected late in the course of the illness. Organisms present in low numbers may be difficult to detect. Polymerase chain reaction is performed on saliva. This is a research procedure. Prior antibiotic therapy or overgrowth of the transport medium with contaminants may result in a negative culture.

Other *Bordetella* - Cultures for *B. parapertussis* and *B. bronchiseptica* are reported 7 to 10 working days after receipt of the specimen.

Reporting of results
Organisms are reported by genus and species.

The results of all specimen requests are reported to the health care provider who submitted the specimen. In addition, the Tennessee Department of Health Communicable and Environmental Disease Services and the health department in the county where the patient lives are sent reports on *Bordetella pertussis*.

Bordetella (Continued)

Criteria for Unacceptable Specimens

All specimens

1. The specimen was not properly identified with the patient's name and/or the tear strip control number.
2. The patient identifier on the specimen did not exactly match the identifier on the form.
3. The specimen or slide was broken in transit.

Clinical

1. Out-dated media or dehydrated media was used.

Reference

1. The specimen was not viable.
2. A mixed specimen was submitted.

Miscellaneous Exam Form PH-1573 FRONT

SOCIAL SECURITY NO.		TENN CARE NO.		MCO		MISCELLANEOUS EXAM		A 349473	
MEDICARE NO.		RECORD FOLDER NO.		DATE REPORTED		DATE/TIME RECEIVED		LAB NO.	
PATIENT'S NAME - LAST, FIRST, MIDDLE		SPOUSE - FIRST NAME		COLLECTION DATE		DATE OF ONSET			
STREET AND NUMBER									
TOWN		STATE		ZIP		SOURCE: <input type="checkbox"/> BLOOD <input type="checkbox"/> URINE <input type="checkbox"/> FECES <input type="checkbox"/> THROAT <input type="checkbox"/> OTHER			
DATE OF BIRTH		RACE		ETHNICITY		SEX		PHONE NO.	
COUNTY NO.		COUNTY NAME		SITE NO.		PATHOGEN/DISEASE SUSPECTED/SYMPTOMS			
						EXAMINATION REQUESTED			
						RECENT TRAVEL 1 <input type="checkbox"/> YES 2 <input type="checkbox"/> NO STATE/COUNTRY			
						EXAMINATION RESULTS			
						<input type="checkbox"/> SUBMITTED TO REFERENCE LABORATORY FOR EXAMINATION. RESULTS FORTHCOMING.			
						EXAMINED BY:			
						RDA-1160			

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TN. DEPT. OF HEALTH DIV. OF LAB SERVICES
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REPORT TO
NAME
ADDRESS
CITY
STATE
ZIP CODE

PH-1573
REV. 11/00

TENNESSEE DEPT. OF HEALTH
LABORATORY SERVICES
MICHAEL W. KIMBERLY, DR. P.H., DIRECTOR

☐ K ☐ J ☐ N
LABORATORY PERFORMING EXAMINATION

BACK

TIGHTEN CAPS SECURELY SUBMIT IN DOUBLE MAILING CONTAINER

STOOL CULTURES FOR SALMONELLA, SHIGELLA, CAMPYLOBACTER, AND E. COLI 0157

Place two (2) cotton tipped swabs dipped into feces or other specimens and insert into Amies, Stuarts, or Cary-Blair transport medium. Submit the transport medium refrigerated within two (2) days of collection.

INTESTINAL PARASITES: Place amount of feces equal to volume of formalin in container designed for intestinal parasites (5% Formalin)

CULTURE FOR IDENTIFICATION - Submit pure cultures on non-selective media such as trypticase soy agar slants or enriched slants (Blood or Chocolate) when required.

ANAEROBIC ORGANISMS - Submit in semi-solid media such as thioglycollate, overlaid with sterile vaspar to prevent exposing culture to oxygen.

TESTING LABORATORY LOCATION CODES

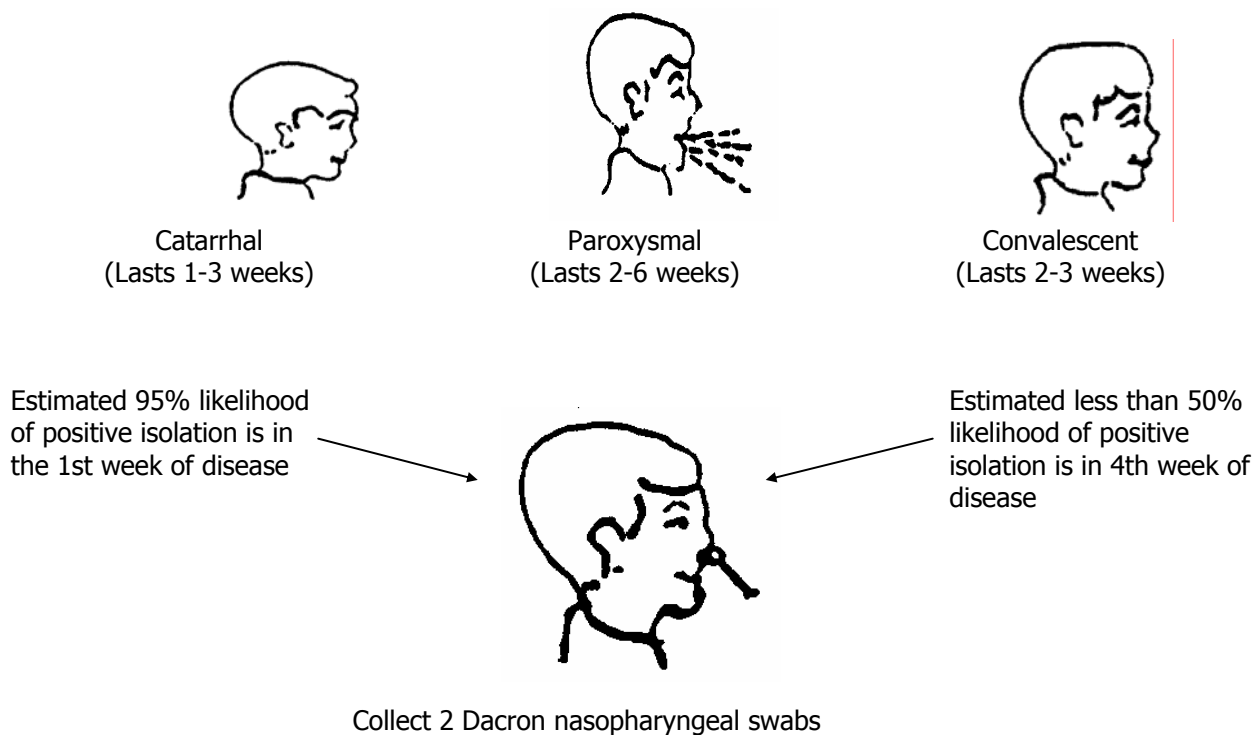
J = JACKSON BRANCH LAB, 295 SUMMAR DRIVE, P.O. BOX 849, JACKSON, TN 38302-0849 - DR ORISTYNE WALKER, DIRECTOR

K = KNOXVILLE BRANCH LAB, 1522 CHEROKEE TRAIL, P.O. BOX 59019, 37950-9019, KNOXVILLE, TN- DR MICHAEL W. KIMBERLY, DIRECTOR

N = NASHVILLE REFERENCE LAB, 630 HART LANE, NASHVILLE, TN 37247-0801 - DR MICHAEL W. KIMBERLY, DIRECTOR

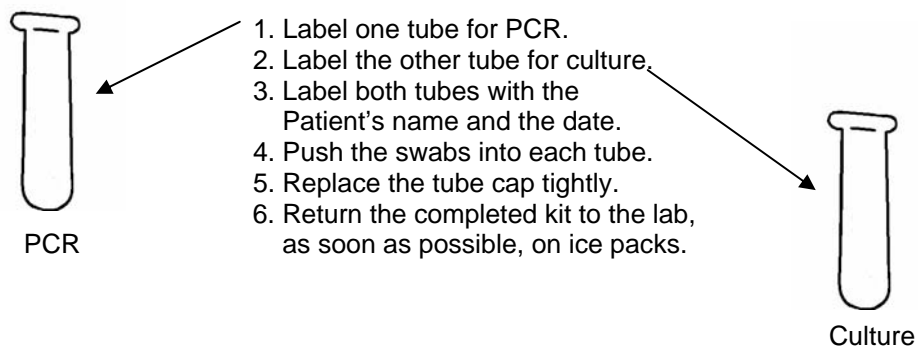
Bordetella (Continued)

Figure II -1
Flow Chart for the Collection of a *Bordetella pertussis* Specimen
Patient in Various Phases. Refer to informational memorandum



Regan-Lowe tube for PCR

Regan-Lowe tube for Culture.



Culture

Botulism (615) 262-6362

Introduction

Botulism is a neuroparalytic disease caused by the toxin of *Clostridium botulinum*. The classical disease is foodborne and results from the ingestion of food in which *C. botulinum* has grown and produced toxin. In rare cases, botulism may also result from the production of toxin by *C. botulinum* growing in a wound. A third type of botulism, referred to as infant botulism, seems to result from ingestion of the organism or its spores. There is evidence that indicates the ingested organisms grow and produce toxin in the infant's gut.

Laboratory Services performs toxin assay tests and procedures for the isolation and identification of *Clostridium botulinum*. When a diagnosis of botulism is considered, the physician or laboratory personnel should call the Tennessee Department of Health (TDH) Epidemiologist at (615) 741-7247. After hours contact the Communicable Disease Consultation Line at (615) 532-8515 or the TDH Laboratory at (615) 262-6300. Epidemiological aid and emergency laboratory services are available 24 hours a day when botulism is suspected.

Serum, feces, vomitus, gastric contents, and pus or wound biopsies are tested when botulism is a possible diagnosis. Suspected foods are tested only when patient testing has resulted in a confirmed case of botulism. Foods are rarely tested in cases of infant botulism. Possible sources of spores for infants are multiple including dust and foods.

The Tennessee Medical Laboratory Act requires that laboratory directors send isolates of *Clostridium botulinum* to the TDH Laboratory for confirmation and surveillance purposes.

Specimen Collection

For clinical specimens and foods -- Refer to Chart II - 2 COLLECTION OF SPECIMENS FOR BOTULISM TESTING.

Culture isolates -- Submit a pure, actively growing culture in a screw-capped tube of liquid or semi-solid medium such as motility medium, thioglycollate, or chopped meat broth.

Specimen Identification

1. Complete **all** the provider and patient information areas on the Miscellaneous Exam Form PH-1573. Include pertinent clinical information with each specimen.
2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Attach the control number on the tear strip to the specimen, and secure it with transparent tape. Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form **will not be tested**.

Botulism (Continued)

Chart II - 2

Collection of Specimens for Botulism Testing

Clinical Diagnosis	Specimen	Amount of Specimen	Test(s) Performed
Foodborne	Serum	10 to 15 ml optimal. 2 ml minimum.	Toxin assay.
	Food	100 gm if available. Do not remove from container.	Toxin assay. Isolation and identification of <i>C. botulinum</i> .
	Feces	100 gm if available, or as much as possible.	
	Gastric contents	100 gm if available.	
	Vomitus	100 gm if available.	
Wound	Serum	10 to 15 ml optimal. 2 ml minimum.	Toxin assay.
	Feces	100 gm if available, or as much as possible.	Toxin assay. Isolation and identification of <i>C. botulinum</i> .
	Pus from wound or biopsied material	Collect in anaerobic collector. Do not refrigerate.	Isolation and identification of <i>C. botulinum</i> .
Infant	Feces	25 gm if available.	Toxin assay. Isolation and identification of <i>C. botulinum</i> .
	Bowel	Use water enema, 20 ml.	

- Collect specimens before antitoxin is given.
- Collect feces, vomitus, and gastric contents in sterile containers. Do not use Cary-Blair transport medium. Ship refrigerated at 2 to 8°C.
- Leave suspect foods in original containers. Leave unopened containers sealed. Ship refrigerated at 2 to 8°C.
- Separate serum from blood cells. Ship refrigerated at 2 to 8°C.
- Collect specimens for wound botulism in an anaerobic collector. Do not refrigerate.

Botulism (Continued)

Shipment of Specimens

1. Serum, food, feces, gastric contents, vomitus, and bowel specimens

Wrap the specimen to cushion it. Place the specimen in a leak-proof, insulated container, and pack with wet ice or freezer packs. Do not pack with dry ice. The specimens should stay cold, but not frozen, until they reach the laboratory. Place the form in a plastic bag to prevent wetting and contamination.

Wound botulism specimens

Place the pus or biopsied specimen from wound botulism in an anaerobic collector. Do not refrigerate.

Reference isolates

Submit a PURE, actively growing culture in screw-cap tube of liquid or semi-solid medium such as motility medium, thioglycollate, or chopped meat broth that has been overlaid with Vaspar *. Ship it in a double-walled mailing container. Do not refrigerate.

2. Affix the mailing label (PH-0838), return address, and infectious substance (etiologic agent) or clinical (diagnostic) specimen label to the outer container.
3. Ship the specimen by the quickest means available to the Tennessee Department of Health Laboratory in Nashville. Suggestions for rapid delivery include courier service, taxi, bus, or plane. Follow the courier's shipping regulations.
4. Notify the Anaerobic Bacteriology Section at (615) 262-6359 as to the method of transportation and when the specimens are scheduled to arrive.

* Vaspar: Melt together equal portions (w/w) Vaseline and paraffin. Dispense in 3 ml amounts and autoclave at 121°C for 30 minutes. Store at room temperature and melt for use as needed.

Reporting Procedures and Interpretation of Results

The TDH Laboratory reports preliminary results to the TDH epidemiologist and the patient's physician by telephone immediately, usually within 24 hours. Communication continues until the testing is complete. Additional specimens may be requested and additional tests performed depending on the patient's condition and laboratory results. A written report is made when all tests are complete.

Results of the toxin tests are reported
Negative for <i>Clostridium botulinum</i> toxin.
Positive for <i>Clostridium botulinum</i> toxin Type ____.

Botulism (Continued)

Results of cultures (original specimen or isolate) are reported
Culture negative for <i>Clostridium botulinum</i> .
Culture positive for <i>Clostridium botulinum</i> , Type ____.

Toxin is identified according to the Centers for Disease Control and Prevention's (CDC) *Laboratory Methods in Anaerobic Bacteriology*. Seven toxigenic types of *C. botulinum* are recognized based on the antigenically distinct toxins produced by the different strains classified in this species (A, B, C, D, E, F, and G). Cases of human botulism are usually associated with toxin Types A, B, and E. Type E is usually associated with foodborne outbreaks involving seafood. Infant botulism is predominately Type A or B.

Isolates are identified according to CDC procedures. Confirmation of species and type is determined by toxin assay tests.

The results of all specimen requests are reported to the health care provider who submitted the specimen, the TDH Communicable and Environmental Disease Services, and the health department in the county where the patient lives.

Criteria for Unacceptable Specimens

1. The specimen was not properly identified.
2. The identifier on the specimen does not exactly match the identifier on the form.
3. The specimen was broken or leaked in transit.
4. The specimen submitted was an improper type.
5. The patient's symptoms do not warrant performance of the test requested.
6. The specimen was submitted improperly.
7. A stool was not submitted refrigerated.
8. A wound culture or isolate was not submitted under anaerobic conditions.
9. A wound culture specimen or isolate was submitted refrigerated.

Botulism (Continued)

Miscellaneous Exam Form PH-1573

FRONT

SOCIAL SECURITY NO.		TENN CARE NO.		MCO	
MEDICARE NO.		RECORD FOLDER NO.			
PATIENT'S NAME - LAST, FIRST, MIDDLE			SPOUSE - FIRST NAME		
STREET AND NUMBER					
TOWN			STATE		ZIP
DATE OF BIRTH		RACE	ETHNICITY	SEX	PHONE NO.
COUNTY NO.	COUNTY NAME				SITE NO.
NAME					
ADDRESS					
CITY			STATE		ZIP CODE
PH-1573 REV. 11/00					
TENNESSEE DEPT. OF HEALTH LABORATORY SERVICES MICHAEL W. KIMBERLY, DR. P.H., DIRECTOR					
<input type="checkbox"/> K <input type="checkbox"/> J <input type="checkbox"/> N					
LABORATORY PERFORMING EXAMINATION					
MISCELLANEOUS EXAM					
DATE REPORTED		DATE/TIME RECEIVED		A 349473 LAB NO.	
COLLECTION DATE		DATE OF ONSET			
SOURCE: <input type="checkbox"/> BLOOD <input type="checkbox"/> URINE <input type="checkbox"/> FECES <input type="checkbox"/> THROAT <input type="checkbox"/> OTHER					
PATHOGEN/DISEASE SUSPECTED/SYMPTOMS					
EXAMINATION REQUESTED					
RECENT TRAVEL 1 <input type="checkbox"/> YES 2 <input type="checkbox"/> NO STATE/COUNTRY					
EXAMINATION RESULTS					
<input type="checkbox"/> SUBMITTED TO REFERENCE LABORATORY FOR EXAMINATION. RESULTS FORTHCOMING.					
EXAMINED BY: RDA-1160					

BACK

TIGHTEN CAPS SECURELY SUBMIT IN DOUBLE MAILING CONTAINER

STOOL CULTURES FOR SALMONELLA, SHIGELLA, CAMPYLOBACTER, AND E. COLI 0157

Place two (2) cotton tipped swabs dipped into feces or other specimens and insert into Amies, Stuarts, or Cary-Blair transport medium. Submit the transport medium refrigerated within two (2) days of collection.

INTESTINAL PARASITES: Place amount of feces equal to volume of formalin in container designed for intestinal parasites (5% Formalin)

CULTURE FOR IDENTIFICATION - Submit pure cultures on non-selective media such as trypticase soy agar slants or enriched slants (Blood or Chocolate) when required.

ANAEROBIC ORGANISMS - Submit in semi-solid media such as thioglycollate, overlaid with sterile vaspar to prevent exposing culture to oxygen.

TESTING LABORATORY LOCATION CODES

J = JACKSON BRANCH LAB, 295 SUMMAR DRIVE, P.O.BOX 849, JACKSON, TN 38302-0849 - DR ORISTYNE WALKER, DIRECTOR
K = KNOXVILLE BRANCH LAB, 1522 CHEROKEE TRAIL, P.O.BOX 59019, 37950-9019, KNOXVILLE, TN- DR MICHAEL W. KIMBERLY, DIRECTOR
N = NASHVILLE REFERENCE LAB, 630 HART LANE, NASHVILLE, TN 37247-0801 - DR MICHAEL W. KIMBERLY, DIRECTOR

Campylobacter
(615) 262-6359

Introduction

Campylobacter are microaerophilic organisms that are identified by morphological and biochemical characteristics. They are small, curved or spiral gram-negative rods with a corkscrew-like motility. *Campylobacter* organisms may be isolated from fecal specimens or blood cultures. Antimicrobial susceptibilities are not performed on these isolates.

Stool specimens for suspected *Campylobacter* infections are tested by the Tennessee Department of Health (TDH) Enteric Bacteriology Section. Refer to ENTERIC BACTERIOLOGY, Section II.

***Campylobacter* illness is a notifiable disease. Complete a Miscellaneous Exam Form PH-1573 with the patient's name and address and send the form to the Enteric Section, TDH Laboratory, Nashville. It is not necessary to send a culture.**

Specimen Collection

Use thioglycollate medium as a transport medium. Pick a single colony to a tube of thioglycollate. Incubate it for 24 to 48 hours until good growth is observed. Overlay with 3/4 inch of sterile Vaspar * to maintain proper atmospheric conditions. Seal the cap with parafilm to prevent leakage. An alternative method involves harvesting the pure growth from a plate with a swab. Inoculate a Cary-Blair and leave the swab in the medium. Send the Cary-Blair refrigerated.

* Vaspar -- melt together equal portions (w/w) Vaseline and paraffin. Dispense in 3 ml amounts and autoclave at 121°C for 30 minutes. Store at room temperature and melt for use as needed.

Specimen Identification

1. Complete all the provider and patient information areas on the Miscellaneous Exam Form PH-1537. Include pertinent clinical and biochemical information with each specimen.
2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Attach the control number on the tear strip to the specimen, and secure it with transparent tape. Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form **will not be tested**.

Shipment of Specimens

1. Thioglycollate Transport Medium

Pack the thioglycollate specimen with absorbent material to prevent breakage and to absorb the fluid if breakage or leakage should occur. Place the form in the outer container.

Cary-Blair Transport Medium

Wrap the Cary-Blair specimen in absorbent material. Place it in a leak proof insulated container and pack with wet ice or freezer packs. Place the form in a plastic bag to prevent wetting or contamination.

2. Affix the mailing label (PH-0838), return address, and infectious substance (etiologic agent) or clinical (diagnostic) specimen label to the outer container.

Campylobacter (Continued)

3. Ship cultures to the Tennessee Department of Health Laboratory in Nashville.
4. Use first-class postage on US mail.

Specimens submitted on plates are acceptable only if they are properly closed in a *Campylobacter* transport bag and delivered by courier to the laboratory.

Reporting Procedures and Interpretation of Results

Most *Campylobacter* cultures are reported within 5 to 7 working days after receipt in the laboratory.

Reporting of Results
Organisms are reported by genus and species.

Organisms are identified to the genus and species level only when culture, morphology, and biochemical test results support the species identification. *Campylobacter* species designations are consistent with the American Society for Microbiology's *Manual of Clinical Microbiology* or according to the *International Code of Nomenclature of Bacteria*.

The results of all specimens are reported to the health care provider who submitted the specimen. In addition, the TDH Communicable and Environmental Disease Services and the health department in the county where the patient lives are sent reports on *Campylobacter* isolates.

Criteria for Unacceptable Specimens

1. The specimen was not properly identified with the patient's name and/or the tear strip control number.
2. The patient identifier on the specimen does not exactly match that on the form.
3. The specimen was broken or leaked in transit.
4. The specimen was non-viable.
5. The specimen was submitted under improper atmospheric conditions.
6. A mixed specimen was submitted.

Enteric Bacteriology (615) 262-6362

Introduction

Feces and other specimens are tested for the presence of enteric pathogens, in particular *Salmonella* Typhi, other *Salmonella* serotypes, *Shigella* species, *Campylobacter jejuni*, and *Escherichia coli* 0157:H7. Testing of clinical specimens for *Yersinia* and *Vibrio* is performed only upon request. Reference specimens are accepted from public and private health care providers for identification and confirmation of members of families of *Enterobacteriaceae* and *Vibrionaceae*. The Tennessee Department of Health (TDH) Laboratory is the serotyping center for *Salmonella* and *Shigella* isolates for the state and participates in the national surveillance programs of the Centers for Disease Control and Prevention (CDC). Isolation and identification techniques include culture procedures, morphological and biochemical characterization, and complete serogrouping and serotyping. Refer to Chart II - 3 ENTERIC TESTING AVAILABLE AT THE TDH LABORATORIES.

Antimicrobial susceptibility testing for patient treatment is not performed in this laboratory.

Feces and other specimens associated with foodborne illness are screened for foodborne disease agents. Refer to **FOODBORNE ILLNESS** in Section II.

The Tennessee Medical Laboratory Act requires laboratory directors to submit pure cultures of the following organisms to the TDH Laboratory: *Escherichia coli* 0157:H7, *Salmonella* species, *Shigella* species, *Vibrio* species, and *Yersinia pestis*.

Patient information only is requested on *Campylobacter* species. You may complete a Miscellaneous Exam Form PH-1573 with the patient's name and address and send the form to the Bacteriology Section, TDH Laboratory, Nashville.

Specimen Collection

Specimens for Routine Screening of Enteric Pathogens: Specimens should be collected early in the course of enteric disease and before antimicrobial therapy is begun. These specimens are routinely cultured for *Salmonella*, *Shigella*, *Campylobacter jejuni*, and *Escherichia coli* 0157:H7. Please indicate if the patient has bloody diarrhea or a suspected *E. coli* 0157:H7 infection. The physician must specifically request *Yersinia* or *Vibrio* for the specimen to be tested for these pathogens.

Collect at least two rectal swabs or swabs of fresh stools from the patient and place the swabs in refrigerated (chilled 1 to 2 hours before use) Cary-Blair transport medium. When obtaining swabs from a patient, first moisten each rectal swab in the holding medium, insert the moistened swab into the rectum 1 to 1 inches, rotate the swab gently, and then return the swab to the same tube of holding medium. Try to ensure that visible fecal material is present on each swab. After obtaining the two fecal swabs, insert both into the same tube of medium and push them to the bottom of the tube. Break off and discard the excess top portion of the swab sticks.

IMPORTANT: Refrigerate or freeze tubes after specimens are placed in them. If the specimens will be tested within 48 hours after collection, they can be refrigerated; however, if the specimens must be held longer than 48 hours, freeze them as soon as possible after they are collected. Although storage in an ultra-low freezer (-70°C) is preferable, storage in a home-type freezer (if it is properly set at -20°C) is acceptable for short periods of time.

Enteric Bacteriology (Continued)

Chart II - 3

Enteric Testing Available at the TDH Laboratories

	Isolation & Preliminary Identification	Identification	Typing/Grouping	Toxin Testing	Other
<i>Aeromonas/Plesiomonas</i>		N			
<i>Campylobacter jejuni</i>	A	N			
<i>Escherichia coli</i> 0157:H7	A	N	N		
<i>E. coli</i> , other		N	¹ CDC		
<i>Salmonella</i>	A	N	N		
<i>Shigella</i> species	A	N	N		
<i>Vibrio cholera</i> & noncholera vibrios	² A	N	N	¹ CDC	
<i>Yersinia pestis</i> (plague)	² N	N			***
<i>Yersinia</i> species (other than <i>Y. pestis</i>)	² A	N			
Other <i>Enterobacteriaceae</i>		N			
Noroviruses (outbreaks only)		N			⁺ PCR

A Tests are performed at the TDH Laboratories in Jackson, Knoxville and Nashville.

N Tests are performed at the TDH Laboratory in Nashville.

¹CDC Centers for Disease Control and Prevention, Atlanta, Georgia.

²Testing of clinical specimens for *Yersinia* and *Vibrio* is performed only upon request.

***Prior consultation with the laboratory is required before sending the specimen. Send by registered mail. This is so that packages can be tracked and undelivered packages may be located quickly. Before shipping, telephone the Enteric Bacteriology Section.

⁺PCR Prior consultation with CEDS requested.

Enteric Bacteriology (Continued)

Reference Cultures: Reference cultures for further identification should meet the criteria for inclusion in the families of *Enterobacteriaceae* or *Vibrionaceae*. Grow a pure culture of the isolated organism on a carbohydrate-free agar slant, such as nutrient agar or trypticase soy agar, for 24 hours.

Specimens for Isolation of *Yersinia pestis* (Plague): Inoculate blood (as least two samples) and aspirated fluids from lymph nodes or bubo into blood culture bottles. Use a sterile container for other specimens, such as sputum, tracheal, bronchial washings, or throat swabs.

Specimens for Norovirus: During an outbreak, stools or feces from a representative sample of 14 persons should be submitted in a Para-paks. Para-paks may be obtained from regional health departments only during outbreaks.

Specimen Identification

1. Complete all the provider and patient information areas on the Miscellaneous Exam Form PH-1573. Include pertinent clinical and epidemiological information for all specimens and biochemical information on isolates. Indicate the organism suspected.
2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Attach the control number on the tear strip to the specimen, and secure it with transparent tape. Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form **will not be tested**.
3. Clinical specimens will not be tested for *Yersinia* or *Vibrio* unless this request is stated on the form and/or the laboratory has been contacted. Contact the laboratory as soon as you know you will submit a specimen for *Yersinia* or *Vibrio* so that appropriate media can be prepared and testing can begin as soon as the specimen is received.

Shipment of Specimens

Ship clinical specimens for routine screening of enteric pathogens under refrigeration to the Tennessee Department of Health Laboratory in Jackson, Knoxville, or Nashville. Ship specimens for isolation of *Yersinia pestis* and all reference cultures to the TDH Laboratory in Nashville.

1. Routine screening specimen – Wrap the specimen in absorbent material to prevent breakage and to absorb the fluid if breakage or leakage should occur. Place it in a leak-proof insulated container and pack with wet ice or cold packs. Place the form in a plastic bag to prevent wetting or contamination. Follow the shipping guidelines of your current carrier or method of shipment. The specimen must be received in the laboratory within 48 hours. If the specimen cannot reach the laboratory within this time, freeze it at -20°C. (Storage at a temperature of -20°C is acceptable for 3 days.) Ship the specimen frozen with ice packs. It must arrive at laboratory in a frozen state.
2. Reference isolates – Pack the specimen with absorbent material to prevent breakage and to absorb the fluid if breakage or leakage should occur. Place it in a double-walled shipping container or the equivalent. Place the form in the outer container. Place the cap on securely. Refrigeration is not required.
3. *Yersinia pestis* specimens – Notify the laboratory before shipping the specimen. Place the specimen in a double-walled shipping container or the equivalent. Pack it with absorbent

Enteric Bacteriology (Continued)

material to prevent breakage and to absorb the fluid if breakage or leakage should occur. Place the form in the outer container. **Contact the Enteric Bacteriology Section before shipping.**

4. Affix the mailing label (PH-0838), return address, and infectious substance (etiologic agent) or clinical (diagnostic) specimen label to the container.
5. Use first-class postage on US mail.
6. When an unusually large number of specimens are anticipated (as in outbreaks), telephone the laboratory before mailing the specimen so that necessary preparations may be made. Notify the laboratory by telephone when one or more specimens for *Yersinia pestis* or *Vibrio cholerae* are being submitted.

Note: **Do not mail specimens on plates.** Specimens submitted on plates are acceptable only if a courier delivers them to the laboratory.

Reporting Procedures and Interpretation of Results

Results from routine screening specimens and from reference specimens are usually reported within 3 to 6 working days after receipt of the specimen. Isolates that are submitted to the Centers for Disease Control and Prevention (CDC) for further testing might require several weeks for identification.

Reporting negative routine screening specimens
No <i>Salmonella</i> , <i>Shigella</i> , <i>Campylobacter jejuni</i> , or <i>Escherichia coli</i> 0157 isolated. All stats are tested for Shiga-toxins to detect other Shiga-toxin producing E. coli.

Reporting enteric pathogens from screening and reference specimens
<p style="text-align: center;"><i>Salmonella</i></p> <p style="text-align: center;"><i>Salmonella</i> serotype _____.</p> <p style="text-align: center;">There are over 2000 known serotypes of <i>Salmonella</i>. <i>Salmonella</i> Typhi is the organism that causes typhoid fever.</p>
<p style="text-align: center;"><i>Shigella</i></p> <p style="text-align: center;"><i>Shigella dysenteriae</i> <i>Shigella flexneri</i> <i>Shigella boydii</i> <i>Shigella sonnei</i></p>

Enteric Bacteriology (Continued)

Reporting enteric pathogens from screening and reference specimens (continued)
<p><i>Escherichia coli</i> 0157:H7</p> <p>Sorbitol negative isolates that agglutinate in 0157 latex reagent are identified biochemically and tested for the H7 antigen.</p>
<p><i>Campylobacter jejuni</i></p> <p><i>Campylobacter jejuni</i> is identified by culture, morphology, and Biochemical means.</p>
Reporting of other isolates
<p>Organisms are reported by genus and species.</p>

Follow-up of cases of salmonellosis and shigellosis and their contacts is recommended following the procedures outlined in the 16th edition of *Control of Communicable Diseases in Man*. Questions concerning follow-up should be addressed to the epidemiologists in the TDH Communicable and Environmental Disease Section at (615) 532-8515.

Organisms are identified to genus, species, and subspecies level when appropriate and only if culture, morphology, and biochemical test results support the identification. Genus, species, and subspecies designations are consistent with designations in the American Society for Microbiology's *Manual of Clinical Microbiology* or according to the *International Code of Nomenclature of Bacteria*.

The results of all specimens are reported to the health care provider who submitted the specimen. In addition, the TDH Communicable and Environmental Disease Services and the health department in the county where the patient lives are sent reports on the following organisms:

Campylobacter jejuni
Escherichia coli 0157:H7
Salmonella Typhi
Salmonella species
Shigella species
Vibrio species
Other Shiga-toxin producing *E. coli*

Criteria for Unacceptable Specimens

All specimens

1. The specimen was not properly identified with the patient's name and/or the tear strip control number.
2. The patient identifier on the specimen does not exactly match the identifier on the form.
3. The specimen was broken or leaked in transit.

Clinical specimens

A provider who submits an unsatisfactory specimen is notified by phone. Even if the specimen is unsatisfactory it is processed, and any pathogen isolated is reported.

If *Shigella* or *Campylobacter* is not isolated, a second specimen must be submitted. (*Shigella* and *Campylobacter* are more dependent on proper transportation conditions for survival than *Salmonella* and *E. coli*.)

1. No preservative was used for transport. If there is a delay of more than 2 to 3 hours after collection, a transport medium is necessary. Cary-Blair transport is available from your local county health department.
2. The specimen was submitted in 5 to 10% formalin.
3. The specimen was not transported under refrigerated (2 to 8°C) or frozen (-20°C) conditions.
4. An unfrozen specimen was received 48 hours after collection.
5. The specimen was resting on top of Cary-Blair transport media. The swab was not preserved by stabbing the Cary-Blair.
6. The specimen contained too much inoculum. A small amount of feces, on a cotton-tipped applicator, should be inserted into the preservative.
7. The Cary-Blair was not inoculated.
8. The Cary-Blair medium had expired.
9. No growth was obtained from the specimen. There was no apparent inoculum.

Reference specimens

1. The specimen was not viable.
2. A mixed specimen was submitted.

Enteric Bacteriology (Continued)

Miscellaneous Exam Form PH-1573

FRONT

SOCIAL SECURITY NO.		TENN CARE NO.		MCO	
MEDICARE NO.		RECORD FOLDER NO.			
PATIENT'S NAME - LAST, FIRST, MIDDLE			SPOUSE - FIRST NAME		
STREET AND NUMBER					
TOWN			STATE		ZIP
DATE OF BIRTH		RACE	ETHNICITY	SEX	PHONE NO.
COUNTY NO.		COUNTY NAME			SITE NO.
REPORT TO					
NAME					
ADDRESS					
CITY			STATE		ZIP CODE
PH-1573 REV. 11/00					
TENNESSEE DEPT. OF HEALTH LABORATORY SERVICES MICHAEL W. KIMBERLY, DR. P.H., DIRECTOR					
<input type="checkbox"/> K <input type="checkbox"/> J <input type="checkbox"/> N					
LABORATORY PERFORMING EXAMINATION					
MISCELLANEOUS EXAM					
DATE REPORTED		DATE/TIME RECEIVED		LAB NO. A 349473	
COLLECTION DATE		DATE OF ONSET			
SOURCE: <input type="checkbox"/> BLOOD <input type="checkbox"/> URINE <input type="checkbox"/> FECES <input type="checkbox"/> THROAT <input type="checkbox"/> OTHER					
PATHOGEN/DISEASE SUSPECTED/SYMPTOMS					
EXAMINATION REQUESTED					
RECENT TRAVEL 1 <input type="checkbox"/> YES 2 <input type="checkbox"/> NO STATE/COUNTRY					
EXAMINATION RESULTS					
<input type="checkbox"/> SUBMITTED TO REFERENCE LABORATORY FOR EXAMINATION. RESULTS FORTHCOMING.					
EXAMINED BY:					
RDA-1160					

BACK

TIGHTEN CAPS SECURELY SUBMIT IN DOUBLE MAILING CONTAINER

STOOL CULTURES FOR SALMONELLA, SHIGELLA, CAMPYLOBACTER, AND E. COLI 0157

Place two (2) cotton tipped swabs dipped into feces or other specimens and insert into Amies, Stuarts, or Cary-Blair transport medium. Submit the transport medium refrigerated within two (2) days of collection.

INTESTINAL PARASITES: Place amount of feces equal to volume of formalin in container designed for intestinal parasites (5% Formalin)

CULTURE FOR IDENTIFICATION - Submit pure cultures on non-selective media such as trypticase soy agar slants or enriched slants (Blood or Chocolate) when required.

ANAEROBIC ORGANISMS - Submit in semi-solid media such as thioglycollate, overlaid with sterile vaspar to prevent exposing culture to oxygen.

TESTING LABORATORY LOCATION CODES

J = JACKSON BRANCH LAB, 295 SUMMAR DRIVE, P.O.BOX 849, JACKSON, TN 38302-0849 - DR ORISTYNE WALKER, DIRECTOR
K = KNOXVILLE BRANCH LAB, 1522 CHEROKEE TRAIL, P.O.BOX 59019, 37950-9019, KNOXVILLE, TN- DR MICHAEL W. KIMBERLY, DIRECTOR
N = NASHVILLE REFERENCE LAB, 630 HART LANE, NASHVILLE, TN 37247-0801 - DR MICHAEL W. KIMBERLY, DIRECTOR

Foodborne Illness (615) 262-6371

Introduction

Testing for foodborne illness is available at the Tennessee Department of Health (TDH) Laboratories in Jackson, Knoxville, and Nashville. Testing for botulism and intestinal parasites, such as *Cryptosporidium* and *Giardia*, is available only at the Nashville Laboratory. The laboratory examines food samples for the presence of disease-producing bacteria only in the case of documented illness under investigation by public health officials.

A foodborne disease outbreak is defined as three or more persons with vomiting or diarrhea who attended the same event or consumed the same meal. Single isolated cases or complaints are not considered outbreaks. EXCEPTION: ONE CASE OF BOTULISM IS SUBJECT TO NOTIFICATION AND INVESTIGATION. Refer to BOTULISM in Section II.

When you suspect a possible foodborne disease, notify your county health department immediately so investigation procedures and sample collection can be started if necessary. Contact your county health department whenever any enteric disease outbreak is suspected in a daycare center, a restaurant, or other facility. Additional assistance in the investigation is available by contacting the TDH Communicable and Environmental Disease Service Section at (615) 532-8515.

Outbreak investigation involves the cooperation of several disciplines within the health department, including the epidemiologist, the county health department, and the laboratory. The investigation requires interviewing patients, collecting food samples and clinical specimens, and laboratory testing. Communication between the various members is essential for the prompt and precise handling of an outbreak.

A wide variety of organisms can cause gastrointestinal illness. Listed below are some of the organisms that are implicated in foodborne outbreaks:

- *Bacillus cereus*
- *Campylobacter jejuni*
- *Clostridium botulinum*
- *Clostridium perfringens*
- *Escherichia coli* 0157
- *Listeria monocytogenes*
- *Salmonella* species
- *Staphylococcus aureus*
- *Vibrio* species
- *Yersinia enterocolitica*

Organisms that can cause an enteric illness outbreak not associated with food include:

- *Cryptosporidium*
- *Giardia*
- *Shigella* species

Foodborne Illness (Continued)

Collection and Shipment of Specimens

The county health department should be notified when an outbreak is suspected. A public health official should conduct an investigation and collect samples following the steps in the Tennessee Department of Health's *Foodborne Disease Outbreak Investigation Manual*.

The laboratory accepts food samples, environmental samples, and clinical specimens as deemed necessary by the investigating official. Clinical specimens should be collected from a representative number of ill persons and an equal number of exposed but well persons.

If there is a danger to the community, the public health official will take action to prevent further spread of the disease.

The **Tennessee Department of Health Laboratories in Jackson, Knoxville, and Nashville** examine specimens in cases of a suspected foodborne illness. Alert the nearest TDH Laboratory when a foodborne illness is suspected so that preparations for handling the food samples and associated specimens can begin.

Complete a Miscellaneous Exam Form PH-1573 for the food samples and an appropriate form for each clinical specimen. Deliver the foods and clinical specimens to the laboratory quickly following the guidelines in the *Foodborne Disease Outbreak Investigation Manual*.

Reporting Procedures and Interpretation of Results

Communications among the TDH public health officials, the TDH Laboratory, and the health care providers are continuous from the time an outbreak is reported until the results are reported. Work-up of specimens requires a constant exchange of information between the laboratory and the epidemiology team. Additional testing is performed as needed.

Examination of food samples requires from 2 to 7 working days depending upon the suspected pathogen. Examination of foods heavily contaminated with *Staphylococcus* may be completed in 48 hours. The presence of low numbers of pathogenic organisms or organisms damaged by processing may take up to 2 weeks for isolation, identification and/or serotyping. Isolation and identification of *Salmonella* species, *Shigella* species, *C. perfringens*, and other more commonly encountered organisms usually require 1 week to identify. Environmental samples and swabs from food handlers are usually reported within 1 to 3 days.

Pulsed field gel electrophoresis for *S. aureus* (from documented outbreaks) is performed on a representative sample of isolates (3-5 isolates) and requires additional time for completion.

Reporting of results of food samples
Positive Results: The name of the pathogen is reported by genus and species. The number of the pathogen per gram is reported if available.
Negative Results: No pathogen isolated.

See individual sections for reporting of results of clinical samples.

Confirmation that a food is involved in an outbreak is made by isolating the same pathogen or toxin in ill patients' specimens and in the implicated food(s). Without clinical specimens, a food can be confirmed as the vehicle of infection if toxins are detected in it. In addition, a food can be epidemiologically suspect if food-specific attack rates are significantly higher in persons who have consumed a food as opposed to those who have not. In addition, confirmation of a foodborne disease can be made if significant numbers of pathogens known to cause food poisoning syndrome are isolated from the food, or if an enteric pathogen such as *Salmonella* or *Shigella* is present in any number.

The results of all specimens and food samples are reported to the health care provider who submitted the specimen. In addition, The Tennessee Department of Health Communicable and Environmental Disease Services and the health department in the county where the patient lives are sent reports if a foodborne illness is detected.

Criteria for Unacceptable Specimens

Unsatisfactory specimens will be assessed on an individual basis.

The specimen should be properly identified, and the specimen identifier should match the information on the form.

Foodborne Illness (Continued)

Miscellaneous Exam Form PH-1573

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PATIENT'S NAME - LAST, FIRST, MIDDLE				SPOUSE - FIRST NAME				DATE/TIME RECEIVED	
STREET AND NUMBER								LAB NO. ▼	
TOWN				STATE		ZIP		COLLECTION DATE	
DATE OF BIRTH				RACE		ETHNICITY		DATE OF ONSET	
COUNTY NO.				COUNTY NAME				SOURCE: <input type="checkbox"/> BLOOD <input type="checkbox"/> URINE <input type="checkbox"/> FECES <input type="checkbox"/> THROAT <input type="checkbox"/> OTHER	
SITE NO.				PATHOGEN/DISEASE SUSPECTED/SYMPTOMS					
				EXAMINATION REQUESTED					
				RECENT TRAVEL 1 <input type="checkbox"/> YES 2 <input type="checkbox"/> NO STATE/COUNTRY					
				EXAMINATION RESULTS					
NAME				<input type="checkbox"/> SUBMITTED TO REFERENCE LABORATORY FOR EXAMINATION. RESULTS FORTHCOMING.					
ADDRESS				EXAMINED BY:					
CITY				STATE		ZIP CODE		RDA-1160	
PH-1573 REV. 11/00				TENNESSEE DEPT. OF HEALTH LABORATORY SERVICES MICHAEL W. KIMBERLY, DR. P.H., DIRECTOR				LABORATORY PERFORMING EXAMINATION	

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